

EXAMINATION OF TYPE MATERIAL OF TWO SPECIES OF *LITOMOSOIDES* (FILARIOIDEA: ONCHOCERCIDAE), PARASITES FROM BATS; TAXONOMIC CONSEQUENCES

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Summary:

Type material of *Litomosoides hamletti* Sandground, 1934 from *Glossophaga soricina soricina* in Brazil and *L. penai* Jiménez-Quirós & Arroyo, 1960 from *Carollia perspicillata azteca* in Costa Rica, was examined. The morphology of the spicules shows that these species belong to the *carinii* group. Their synonymy with *L. guiterasi* Pérez Vigueras, 1934, from *Artibeus jamaicensis yucatanicus* in Cuba, does not appear justified because they are distinct in several characters (body length, width of female, size and shape of buccal cavity and capsule, shape of right spicule). *L. hamletti* is a valid species; *L. penai* is closely related to it and is considered to be a sub-species, *L. hamletti penai* Jiménez-Quirós & Arroyo, 1960. The material recovered from *Glossophaga* spp., previously assigned to *L. guiterasi* by several authors, is identified as *L. h. hamletti*. *L. guiterasi* appears to be closely related to *L. chandleri* Esslinger, 1973; *L. chitwoodi* n. sp. (= *Litomosoides* sp. Chitwood, 1938) seems close to these species; all three are parasites of *Artibeus* spp.

KEY WORDS : filariae, *Litomosoides* spp., type material, *L. hamletti*, *L. penai*, bats.

Résumé : ÉTUDE DE MATÉRIELS TYPES DE DEUX ESPÈCES DE *LITOMOSOIDES* (FILARIOIDEA : ONCHOCERCIDAE) PARASITES DE CHAUVESOURIS; CONSÉQUENCES TAXONOMIQUES

Les matériels types de *Litomosoides hamletti* Sandground, 1934, parasite de *Glossophaga soricina soricina* au Brésil et de *L. penai* Jiménez-Quirós & Arroyo, 1960, parasite de *Carollia perspicillata azteca* au Costa Rica, sont examinés. Leurs spicules montrent qu'elles appartiennent au groupe *carinii*. Leur synonymie avec *L. guiterasi* Pérez Vigueras, 1934, parasite d'*Artibeus jamaicensis yucatanicus* à Cuba, n'apparaît pas justifiée car elles se distinguent de cette espèce par plusieurs caractères (taille du corps, largeur de la femelle, taille et forme de la capsule et de la cavité buccales, forme du spicule droit). *L. hamletti* est une espèce valide; *L. penai* en est très proche et considéré comme une sous-espèce, *L. hamletti penai*. Les matériels récoltés chez *Glossophaga* spp., qui avaient été rapportés à *L. guiterasi* par plusieurs auteurs, sont identifiés à *L. h. hamletti*. *L. guiterasi* s'avère très proche de *L. chandleri* Esslinger, 1973; *L. chitwoodi* n. sp. (= *Litomosoides* sp. Chitwood, 1938) semble proche de ces espèces; elles sont parasites d'*Artibeus* spp.

MOTS CLÉS : filaire, *Litomosoides* spp., matériel type, *L. hamletti*, *L. penai*, chauvesouris.

INTRODUCTION

Identification of *Litomosoides* species from bats is often difficult, because diagnostic criteria are not well defined for several species. The problem treated here is that of *L. guiterasi* (Pérez Vigueras, 1934) and two species often considered as synonymous, *L. hamletti* Sandground, 1934 and *L. penai* Jiménez-Quirós & Arroyo, 1960.

The type host of *L. guiterasi* is *Artibeus jamaicensis parvipes* Renn, from Cuba; its description is imprecise. Redescriptions have been made with specimens recovered from *Glossophaga soricina* Pallas from Brazil

(Rêgo, 1961a, b), from Colombia (Esslinger, 1973) and, recently, from Venezuela (Guerrero *et al.*, 2002). However these last authors noticed that the redescrptions, although agreeing with each other, did not fit well with the original description considering the size of the specimens and the form of the buccal capsule.

The type host of *L. hamletti* is *G. soricina* from Brazil. Sandground compared his material with specimens sent by Pérez Vigueras and he proposed a new taxon "only provisionally". Chitwood (1938) identified as *L. hamletti* a male from *G. soricina leachii* Gray from Yucatan. Later the taxon was synonymized with *L. guiterasi* by Rêgo (1961); this was accepted by Esslinger (1973) but not by Bain *et al.* (1989) because the spicules of the taxa were differently figured by Sandground. These authors had defined two morphological groups among *Litomosoides* from rodents, marsupials and Chiroptera, the *carinii* and the *sigmodontis* groups, distinguished by the spicules; they placed *L. guiterasi* in the first group and *L. hamletti* in the second.

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The type host of *L. penai* is *Carollia perspicillata azteca* (Saussure) (= *Hemiderma perspicillata azteca*) from Costa Rica. The filarial species was synonymized with *L. guiterasi* by Rêgo (1961b).

We have examined the type material of *L. hamletti* and *L. penai*, but we have not yet succeeded in obtaining Pérez Vigueras' material. Nevertheless, our observations allowed a more precise analysis of Pérez Vigueras' description and thus clarified the status of these species. The taxonomic consequences extend beyond these three taxa.

MATERIALS AND METHODS

The morphological characters of the filariae were studied as described by Notarnicola *et al.* (2000) and Guerrero *et al.* (2002). The buccal ratio is the length of the buccal capsule divided by its maximal external diameter, and the caudal ratio is the tail length divided by its width at the anus. In this study, the microfilariae extracted from the uteri of fixed females (*L. penai*) were stained by adding a drop of the diluted vital stain Meldolan Blue to lactophenol (Bain & Guerrero, 2003), to better visualize the nuclei and the sheath. Length of adults is given in mm, all other measurements are in μm ; they are presented in Tables I-III.

The names of the authors of the new taxon are Bain, Guerrero & Rodríguez.

RESULTS

TYPE MATERIAL OF *LITOMOSOIDES HAMLETTI* SANDGROUND, 1934 (Fig. 1; Table I)

Type Host: *Glossophaga soricina soricina* (Pallas), Phyllostomidae.

Infection site: Peritoneal cavity.

Type locality/collection dates: Arapua, Matto Grosso, Brazil, December 1933.

Specimens deposited in the U.S. National Collection, storage number T42-A.

The type material comprised two males and five females (Sandground, 1934). The males are still present; one is in an excellent state of preservation despite the beginning of a host reaction, as indicated by the presence of groups of cells attached to the worm cuticle (Fig. 1A); the second male has been affected by this reaction and its caudal papillae are no longer identifiable. There remain three fragments of the females, an anterior altered region, of which the buccal cavity and buccal capsule are uniformly cuticularized, a caudal region in good condition, and a median, very damaged, fragment.

The buccal cavity and the buccal capsule conform to Sandground's (1934) description: narrow tubular cavity, much longer than wide; thin buccal capsule, with a slightly prominent ring midway along the capsule; the anterior part of the capsule is more transparent because

Female	Sandground 1934	Present study type material	Male	Sandground 1934	Present study type material	Chitwood, 1938 Yucatan material
Length	35-51	?	Length	15-16.5	16.8	14.9
Max. width	170	140	Max. width	100-110	115	125
Buccal capsule	21	21	Buccal capsule	19	20	20
Oesophagus	690-780	?	Oesophagus	450-590	595	
Vulva	400-450	330	Tail	57	65	60
Tail	101	93	Left spicule (handle)	220-280	225 (135)	220 (145)
Lateral bosses	?	absent	Right spicule	82-90	73	72
						63

Table I. – Measurements of *Litomosoides hamletti* Sandground, 1934, type material and material of Chitwood (1938).

Female	Jiménez Q. & Arroyo 1960)	Present study						Male	Jiménez Q. & Arroyo 1960	Present study
Length	71.2-76.5	71*	70	60	73	66.6	fragments	Length	15.04-17.49	16.7**
Max. width	227	205	185	200	210	210	160-210	Max. width	143	128
Buccal capsule	28-35	28	21	24	27	26	20.5-26	Buccal capsule	28-35	21
Oesophagus	701-703	600	660	600	650	760	580-650	Oesophagus	701-703	550
Vulva	726	560	640	870	650	480	650-750	Tail	65	78-80
Tail L/w	774/113	150	150	100	120		140-180	Left spicule (handle)	180-199	230 (140)
Lat. bosses		no	no	no	no	no	no	Right spicule	55-59	80-70
Nerve ring		230	230	210	200	190		Area rugosa length	?	550-1800

* holotype ; ** allotype.

Table II. – Measurements of *Litomosoides penai* Jiménez-Quirós & Arroyo, 1960, type material.

less sclerotized, as in all *Litomosoides* spp.; buccal ratio 3. Thick oesophagus with a uniform structure, without glandular differentiation. Head papillae were identified on the male in good condition: four external labial papillae very close to the apex, a single cephalic papilla and two amphids.

The anterior part of the testis is thick and its apex lies at the mid-point of the oesophagus. The male tail in lateral view conforms to Sandground's (1934) illustration: short (ratio equal to 2), straight, with round extremity and two pairs of papillae (male 1); in ventral view (male 2), the tail is seen to be conical; phasmids are divergent and no cuticular ornamentation is observed near their apex. The left spicule has a handle longer than the blade, as figured by Sandground (1934); the blade is a narrow simple membrane, supported by a long thin cuticular axis and the opposite side is thickened only proximally; the distal part of the blade, being only membranous, is less easy to identify inside the worm. The right spicule in Fig. 4 of Sandground (1934) seems to have a membranous distal half but it is in fact strongly sclerotized; this part comprises an angular dorsal heel, followed by a segment twice as long as wide, flat at the anterior aspect, slightly convex at the posterior aspect, and a truncated extremity with a small ventral crest. The area rugosa is present and composed, as in other *Litomosoides* species, of transverse bands of short longitudinal crests.

In the female the oesophageal-intestinal junction was not identified; the vulva is not far from the apex, as in the original description; the vagina is subspherical. The female tail, which is attenuated in Sandground's (1934) figure, is almost cylindrical in the remaining specimen, with wide, almost flat, extremity; the caudal ratio is 1.7; there is a small cuticular point close to the apex of the phasmids.

TYPE MATERIAL OF *LITOMOSOIDES PENAI*
JIMÉNEZ-QUIROS & ARROYO, 1960 (Fig. 2, Table II)

Type Host: *Carollia* (previously *Hemiderma*) *perspicillata azteca* (Saussure), Phyllostomidae.

Infection site: Peritoneal cavity.

Type locality/collection dates: Santa Ana, San Jose, Costa Rica.

Specimens deposited in Departamento de Parasitología, Facultad de Microbiología, Universidad de Costa Rica; collection number 3-4.

The type material initially comprised 15 specimens (Jiménez-Quirós & Arroyo, 1960). It is still rich, but examination showed it to be heterogeneous. A long anterior fragment and a long posterior fragment of female are identified as *L. brasiliensis* Lins de Almeida, 1936 by their dimensions and the characters which were recently described by Guerrero *et al.* (2002): buccal

capsule with two rings, the posterior of which is more developed, long oesophagus, vulva very posterior to the oesophageal-intestinal junction, elongated vagina, very long, cylindrical tail, phasmids close to the ventral line and decorated with two round points. Measurements: body 210 µm wide, buccal capsule 23 µm long, oesophagus 850 µm long, nerve ring and vulva 580 and 2,850 µm from apex, respectively, tail 450 µm long.

The remainder of the material comprises one male, four complete females, and three anterior and three posterior parts of females which fit with the original description. A fourth posterior region of a female has a clearly longer tail (280 µm); this fragment cannot be identified and is not considered further in this morphological analysis.

Long narrow buccal cavity, as in the original description; long thin buccal capsule usually with a slightly prominent ring (absent from the single male), placed at the mid-point of the capsule; anterior segment of capsule slightly cuticularized, often thickened at its junction with the sclerotized posterior part; buccal ratio 3-4. Head papillae observed on three female specimens as follows: four anterior external labial papillae, two amphids and a single cephalic papilla, placed in a small submedian (ventral) notch; this papilla may be divided into two round protrusions. Oesophagus with slight glandular differentiation in the posterior half.

Female. Vulva at level of mid-length of oesophagus ($n = 1$), or close but anterior to the oesophageal-intestinal junction ($n = 3$) or posterior to it ($n = 3$); subspherical vagina. Tail conical with rounded extremity. Male. Thick anterior part of testis with the apex at the level of the oesophagus. Finger-shaped tail, ventrally flexed; caudal ratio 2.5. Caudal papillae: the original description indicated three pairs of papillae as a constant character; the remaining specimen has five papillae; that closest to the cloacal aperture is situated on the right side and the others are paired. The spicules are like those of the type material of *L. hamletti*.

The microfilariae, not described previously, present the characters of the other species of *Litomosoides*: a sheath, the body attenuated at both extremities and a prominent cephalic hook. They are small: 53, 60, 70 and 72 µm long and 3.5-4 µm wide; caudal extremity with a terminal nucleus.

DISCUSSION

Examination of the material of Sandground (1934) and of Jiménez-Quirós & Arroyo (1960) enabled us to compare them with the description of *L. guiterasi*, with which they have been synonymized.

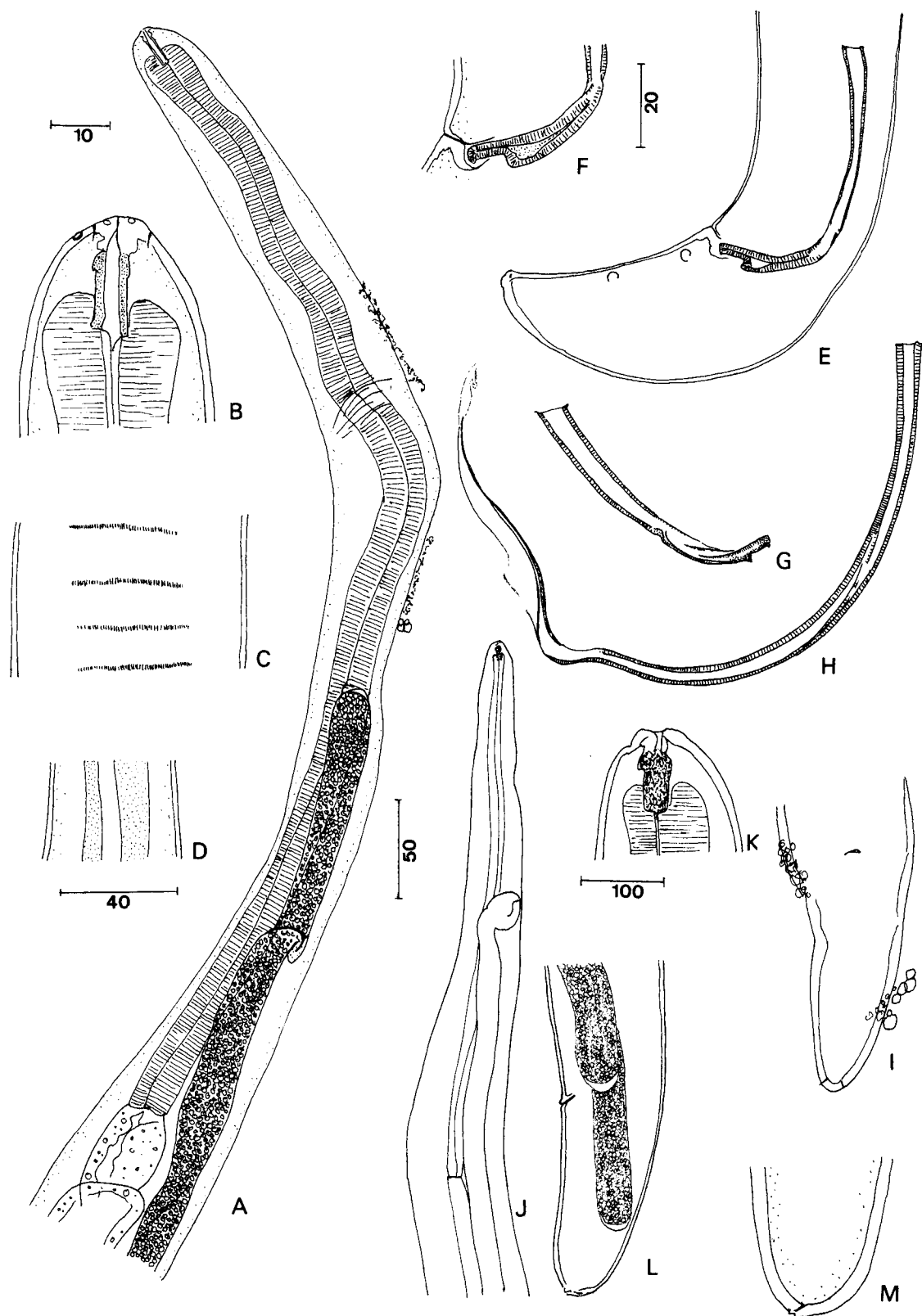


Fig. 1. – Type material of *Litomosoides b. bamletti* Sandground, 1934. A to I, males. A, anterior region, left lateral view (a few host cells are attached on the cuticle). B, head, median view. C, area rugosa. D, lateral chord and lateral thickening at level of nerve ring, lateral view. E, male tail and right spicule, left lateral view. F, distal region of right spicule, left lateral view. G, the other male, right spicule, right lateral view. H, left spicule of the male shown in A, left lateral view. I, tail, ventral view, second male (host cells are attached). J to M, female. J, anterior region, right lateral view. K, abnormally sclerotized buccal cavity and capsule. L, tail, left lateral view. M, caudal extremity, left lateral view. Scale bars: A, L, 50 µm; B, F, 10 µm; C, E, G, H, K, I, M, 20 µm; D, 40 µm; J, 100 µm.

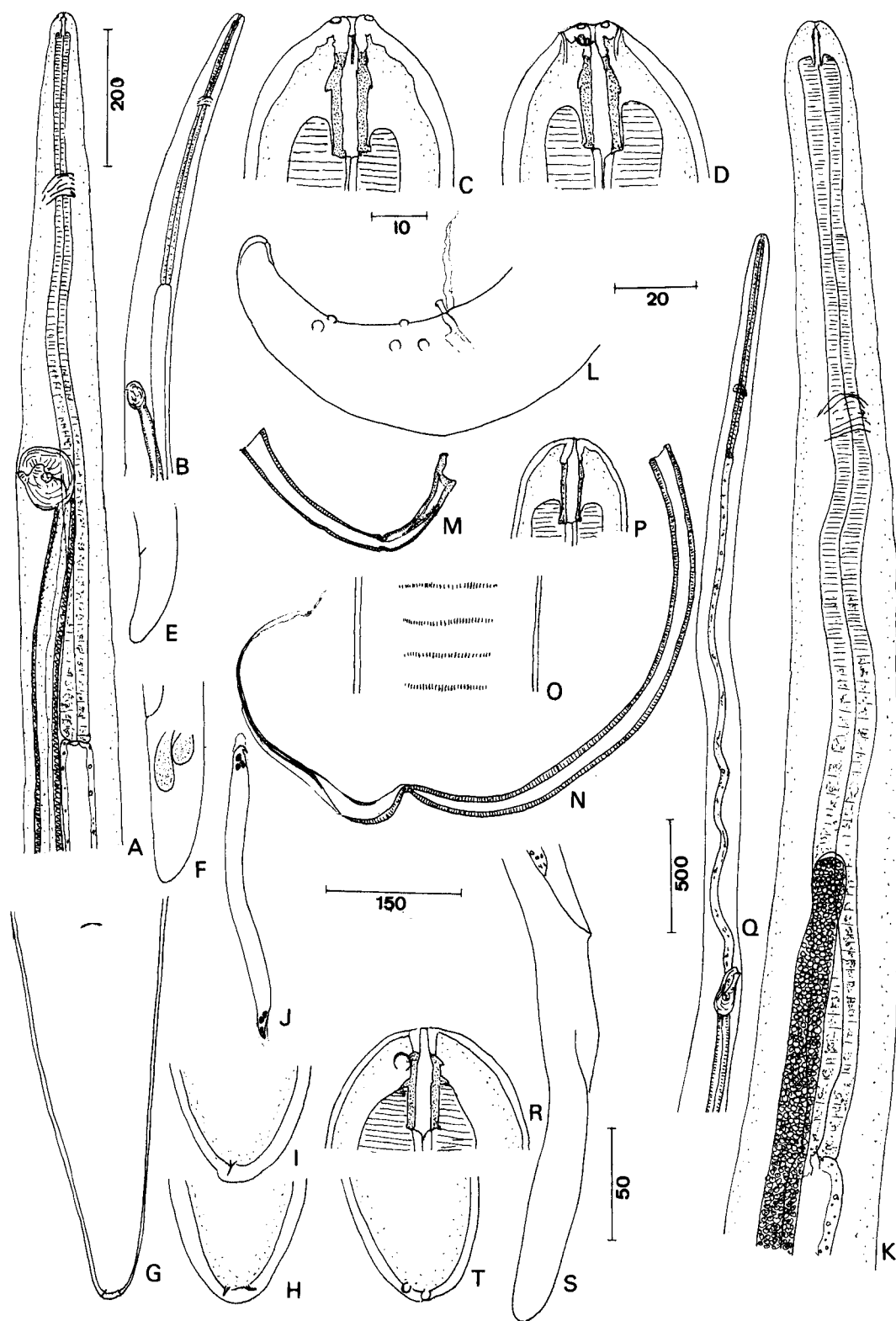


Fig. 2. – Type material of Jiménez-Quirós & Arroyo, 1960, including *Litomosoides hamletti penai* and a female of *L. brasiliensis*. A to G, *L. b. penai*, female. A & B, anterior region showing extreme positions of vulva, left lateral view. C & D, head of two specimens, respectively lateral and ventral views. E & F, tail of two specimens, left lateral view (apex of ovaries indicated on F). G, other specimen, tail, ventral view. H & I, other specimen, caudal extremity, lateral and ventral views. J, uterine microfilaria. K to P, *L. b. penai*, male allotype. K, anterior region, median view. L, tail, left lateral view. M, right spicule, right lateral view. N, left spicule, left lateral view. O, area rugosa, ventral view. P, buccal capsule. Q to T, *L. brasiliensis*, female. Q, anterior region, lateral view. R, buccal capsule, left lateral view (a cephalic papillae is identified). S, tail, right lateral view. T, caudal extremity, ventral view. Scale bars: A, E, F, S, 150 µm; B, 200 µm; C, D, J, 10 µm; G, T, 50 µm; I, H, L, M, N, O, P, R, 20 µm; Q, 500 µm.

<i>guiterasi</i> Rêgo 1961a	<i>guiterasi</i> Esslinger 1973	<i>guiterasi</i> Guerrero <i>et al.</i> 2002	Original taxa Author Date	<i>guiterasi</i> Pérez Viguera 1934	<i>chandleri</i> Guerrero <i>et al.</i> 2002	<i>chandleri</i> Esslinger 1973	<i>Litomosoides</i> sp. Chitwood 1938
Male							
10.72-16.75	10-15	13.4-17.6	Length	8	8.6-10.2	7.4-10.2	?
100-130	83-100	98-115	Max. width	104 (1.04*)	85-117	83-102	?
16-33	20-29	19-26	Buccal capsule	12.5	12-14.4	13.5-15.3	?
520-650	480-583	450-656	Oesophagus	515	450-500	416-550	?
50-59	56-67	48-68	Tail	80	90-105	81-99	?
185-197	180-202	195-245	Left spicule	200	190-255	199-220	?
63-80	59-81	71-85	Right spicule	60	56-69	46-62	?
Female							
40.2-52.93	29-47	40.3 & 51.3	Length	24	19.3-24	14.5-22	15
610-830*	139-189	162 & 180	Max. width	225 (2.25*)	170-280	168-270	150
21-25	24-27	23 & 28.5	Buccal capsule	13 (20*)	14.5-16.8	13-16.3	12
610-830	515-717	600 & 700	Oesophagus	424	450-690	410-632	583
350-670	350-485	509 & 507.5	Vulva	420	540-710	460-555	810
121-130	114-143	134.5 & 135	Tail	subterminal*	195-285	177-245	583
<i>b. hamletti</i>	<i>b. hamletti</i>	<i>b. hamletti</i>	Present taxa	<i>guiterasi</i>	<i>chandleri</i>	<i>chandleri</i>	<i>L. chitwoodi</i> n. sp.
<i>Glossophaga</i> <i>soricina</i> , <i>G. sp.</i>	<i>Glossophaga</i> <i>soricina</i>	<i>Glossophaga</i> <i>soricina</i>	Hosts	<i>Artibeus</i> <i>jamaicensis</i> <i>parripes</i>	<i>Artibeus</i> <i>jamaicensis</i> <i>A. planirostris</i>	<i>Artibeus</i> <i>jamaicensis</i>	<i>Artibeus</i> <i>jamaicensis</i> <i>yucatanicus</i>
Bra. Sao P. Man. Amaz. Brasil	Valle Colombia	Miranda Venezuela	Region Country	Cuba	Mir. Nou. Madre Ven. Fr Gu. Peru	Valle Colombia	Yucatan Mexico

*Data very likely inaccurate.

Table III. – Published data on *L. guiterasi*, *L. chandleri* and *Litomosoides* sp. with the identification proposed in this study.

The description of *L. guiterasi* by Pérez Viguera (1934) contains a few errors, some easy to detect, some more subtle. The subterminal position of the anus in the female is very likely a mistake, considering that all *Litomosoides* species have a long tail in the female; however, other characters show that this material is quite distinct from those described above: the male is short (8 mm instead of 10-17 mm), the female is also short (24 mm) and, in contrast, thick (225 µm wide [not 2.25 mm, as given erroneously in the description]), equal to or greater than the width of the females of the other material (Table III). The buccal capsule, despite a not very coherent description, appears also to differ: the measurements given for the female are 20 µm long and 6.5 µm wide; however, in the figure of Pérez Viguera, which is reproduced here (Fig. 3), the capsule is only twice as long as wide, making it 13 µm long; its length then fits with that of the male buccal capsule (12 µm long); this is more likely because the size of the capsules differs very slightly between the sexes (Esslinger, 1973; Bain *et al.*, 1980; Bain *et al.*, 1989; Moraes-Neto *et al.*, 1997; Notarnicola & Navone, 2002).

Consequently, *L. hamletti* is a valid species. We observed that the morphology of its adults, including the dimensions, cannot be distinguished from that of the specimens described later under the name *L. guiterasi*

by Rêgo (1961a, b), Esslinger (1973) and Guerrero *et al.* (2002) (Table III). The microfilariae, the morphology of which is an important specific character, remain undescribed in the type material of *L. hamletti*. They are presently known only in the Colombian and Venezuelan material; they present slight differences which could be explained by the different techniques of fixation (Guerrero *et al.*, 2002). We thus think that, at present, no solid criterion distinguish *L. guiterasi sensu* Rêgo (1961a), Esslinger (1973) and Guerrero *et al.* (2002) and we identify these materials as *L. hamletti*. The material of Jiménez-Quirós & Arroyo, excluding the specimen of *L. brasiliensis*, resembles *L. hamletti*: similar buccal capsule, spicules and head papillae. However, it presents a few peculiarities (Tables I & II): the female body is 60-73 mm long whereas it does not exceed 53 mm in the diverse materials of *L. hamletti* cited above; the vulva is more often close to the oesophageal-intestinal junction or even posterior to it (3/7 females); the female tail is attenuated, with a round, instead of a truncated, extremity (Rêgo, 1961; Esslinger, 1973; Guerrero *et al.*, 2002). We propose subspecific status for this material, *L. hamletti penai*. A female holotype and a male allotype are designated among the other type specimens (Table II).

The present analysis also reveals the similarities of *L. guiterasi* with a species described from *Artibeus*

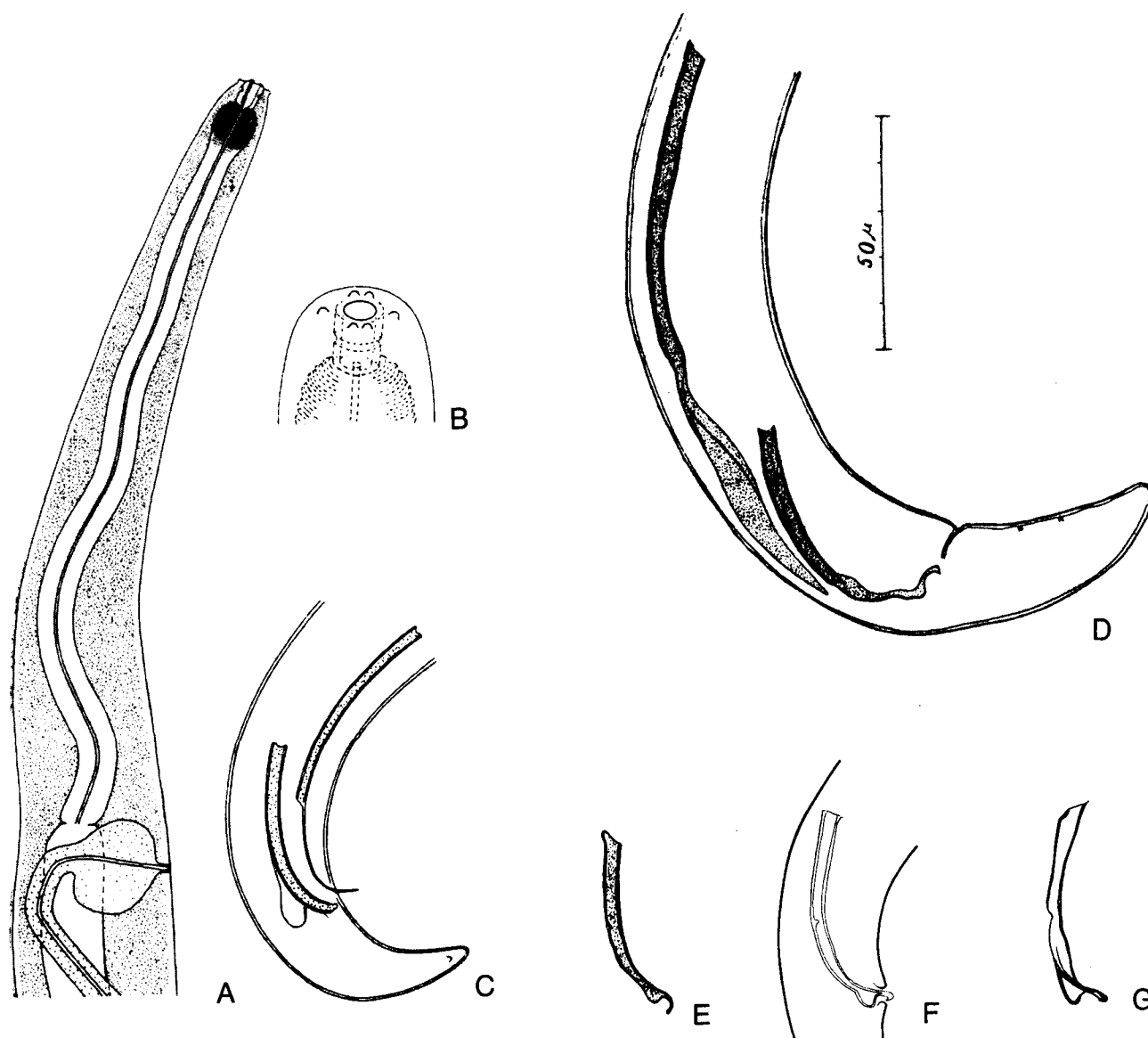


Fig. 3. – *L. guiterasi*, *L. hamletti* and *L. chandleri*, from published figures. A to C, *L. guiterasi* from Pérez Vigueras (1934). A, anterior region of female, right lateral view. B, head. C, male tail, right lateral view. D, *L. hamletti* from Sandground (1934), male caudal region. E, *L. guiterasi*, right spicule, from Sandground (1934). F, Right spicule of *L. chandleri* from Esslinger (1973). G, Right spicule of *L. chandleri* from Guerrero *et al.* (2002). Scale bar: D, 50 µm ; A, C, oesophagus and right spicule are 424 µm and 60 µm, respectively (Pérez Vigueras and Table III); E, F, G, right spicule is 60–65 µm long.

jamaicensis in Colombia, *L. chandleri* Esslinger, 1973. These two species are short and wide, with a buccal ratio of about 2, a vulva situated at the oesophageal-intestinal junction, a subspherical vagina, and a left spicule with its handle longer than the blade; these last three characters are shared with *L. b. hamletti* and *L. b. penai*. The right spicule of *L. guiterasi* was not precisely drawn by Pérez Vigueras but it was well represented by Sandground (1934) and both drawings show the same morphological particularity: the subterminal dorsal heel is very prominent and rounded (Fig. 3); a similar shape was observed in *L. chandleri* by Esslinger (1973) and by Guerrero *et al.* (2002) (Fig. 3).

It is necessary to mention *Litomosoides* sp. Chitwood, 1938, known only by one female and recovered in Mexico from *Artibeus jamaicensis yucatanicus* (Allen), because this species seems to be close to the two previous species: it has a 12 µm long buccal caspule and cuticular ornamentation on the lateral line, described by Esslinger (1973), which is similar to that of *L. chandleri*. But *Litomosoides* sp. has a very long tail (583 µm) and the vulva is clearly post-oesophageal. We propose to name this material *L. chitwoodi* n. sp. Chitwood (1938) did not create a new taxon because he judged the buccal capsule a “structure too variable for serious consideration”. We have shown that this variability in

fact results from a mixture of species. Our experimental work on two *Litomosoides* species from rodents has also confirmed the diagnostic value of this character: the capsule is identical in both the natural and surrogate hosts, the jird *Meriones unguiculatus* and laboratory mice (Maréchal *et al.*, 1993).

CONCLUSION

The species from bats studied here belong to the *carinii* group of *Litomosoides*. They resemble in the position of the vulva, shape of the vagina, and size of the spicules. By redefining the morphology of type specimens of two species, this study has revealed an association between these *Litomosoides* species and certain genera of phyllostomid bats. The three species which seem closely related, *L. guiterasi*, *L. chandleri* and *L. chitwoodi*, are parasites of *Artibeus* species. *L. b. hamletti* is linked to *Glossophaga* species, and a slight evolutionary development, represented by *L. b. penai*, occurred with the passage to another host in Central America, *Carollia perspicillata azteca*.

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